Chapter

Antimicrobial Potential of Pomegranate Extracts

Vildan Celiksoy and Charles M. Heard

Abstract

The search for plant extracts with efficacious antimicrobial activity remains important, partly due to fears of the side effects associated with conventional antibiotics and to counter the emergence of resistant microorganisms. Pomegranate extracts have been used for millennia for their anti-infective properties, with activity more recently being attributed to its rich composition of ellagitannins and other secondary polyphenolic compounds. This chapter highlights the growing number of publications that have probed the activity of pomegranate extracts against microbes. Research generally supports folklore claims and has shown that pomegranate extracts possess unusual and potent broad-spectrum activities against Gram-positive and Gram-negative bacteria (planktonic and biofilm), fungi, viruses and parasites. Possible pathways/mechanisms of antimicrobial activity of pomegranate extracts are discussed and enhancement/potentiation of such activity using metal ions considered.

Keywords: antimicrobial, bacteria, fungi, viruses, parasites, polyphenols, pomegranate extracts, biofilm, tannins, punicalagin

1. Introduction

Infectious diseases caused by pathogenic microbes are a fundamental problem and remain one of the major factors behind high morbidity and mortality across the world, especially in developing countries. This is exacerbated by the worldwide emergence of antibiotic-resistant pathogens which has in turn given increased urgency to the discovery of new antimicrobial compounds, including those derived from plants [1, 2].

The pomegranate, fruit of the *Punica granatum* L. tree, is one of oldest recorded edible fruits and it has been used as a folklore medicine since ancient times. There are records of it being used to treat inflammatory diseases and disorders of the digestive tract in the Ayurvedic and Unani systems [3, 4]. In terms of infections, the ancient Egyptians used it in the treatment of tapeworms and other parasites [5], whereas other cultures have used pomegranates to treat diarrhea and dysentery [6–8], although at the time they would not have known that pathogenic microbes were responsible. In more recent times, the pomegranate has been extensively and scientifically studied for its antimicrobial potential in a diversity of areas such skin infections, dentistry, food preservation etc. [9].

The phytochemistry of pomegranate extracts is well described in the literature [10–12] and they are known to be rich in bioactive compounds especially

polyphenolics including anthocyanins and ellagitannins, in particular punicalagin, which is in the highest proportion [13]. As will be seen, it has become apparent that the pomegranate possesses unusual broad-spectrum potency against a wide range of species, which generally correlates with its polyphenol concentration.

In this chapter we aim to summarise published research into pomegranate extracts as antimicrobials and discuss some of the purported mechanisms behind such activity. Finally, the enhancement of antimicrobial activity by co-administration with metal ions is considered.

2. Activity against bacteria

Staphyllococcus aureus (*S. aureus)* and methicillin resistant *Staphyllococcus aureus* (MRSA) have received the greatest attention as targets for pomegranate extract activity. In 2010, the antibacterial activity of crude and purified extracts of pomegranate peel were assessed by Panichayupakaranant *et al.* 8 mg crude peel loaded discs showed 20 mm and 30 mm zone of inhibition against clinical isolates of *S. aureus* and *E. coli*, respectively. The purified peel extract discs, loaded up to 8 mg, exerted a range of zones of inhibition between 15-20 mm for *S. aureus* and 20-30 mm for *E. coli*. Using standardized peel extract, minimum inhibitory concentrations (MIC) values of 0.016, 0.008, and 0.008–0.016 mg/mL were obtained for *S. aureus*, *S. epidermidis* and *Propionibacterium acnes* respectively. Tetracycline was used as a positive control in this study and standardized pomegranate rind extract showed lower activity in zone of inhibition assays, with tetracycline also showing a lower minimum inhibitory concentration (MIC) [14]. A methanolic extract of pomegranate peel inhibited biofilm formation and eradicated pre-formed biofilm of *S. aureus*, MRSA, *E. coli* in the concentration range 25 to 150 μg/mL [15]. In the same study, ellagic acid showed biofilm inhibition and eradication activity at somewhat lower concentrations (5–40 μg/mL) than pomegranate peel extract. Furthermore, while pomegranate extract was able to inhibit the growth of *S. aureus*, it also suppressed enterotoxin production [5].

Pomegranate extracts have shown antimicrobial activity against to a range of oral microbes. It has been found that pomegranate extract powder at 1 mg/mL was effective against primary and secondary colonizer bacteria of dental plaque: *F. nucleatum, P. gingivalis, P. intermedia, S. mutans* and *A. actinomycetomomitans* [16]. In another *in vitro* study, pomegranate alcoholic extracts have been tested on bacteria which are collected from patients who have tooth decay or periodontitis and inhibited a range of bacteria in both planktonic and biofilm conditions [17]. Synergistic bactericidal activity against *S. mutans* and *R. dentocariosa* was reported for pomegranate extract in combination with other plant polyphenolic extracts, honey and myrtle [18].

Moreover, 'standardized' pomegranate peel extract showed higher antimicrobial activity than other parts of pomegranate (flower, leaf, stem) and ciprofloxacin (2 mg/mL) against *S. mutans, Salmonella mitis* and *L. acidophilusin* in a zone of inhibition assay [19]. Again, pomegranate gel showed an inhibitory activity against *S. mutans, Salmonella sanguis,* and *S. mitis* [20]. This gel also showed antiadhesive activity against *S. mutans* and *S. mitis* at lower than minimum inhibitory concentrations to a glass surface. In addition to inhibition activity on bacterial growth and biofilm, pomegranate extracts showed antiadhesive activity for *S. mutans* adherence on tooth surface in orthodontic treated patients [21]. In other clinical studies, the antiplaque effect and prophylactic benefits of pomegranate have been highlighted [22]. Recently, a systematic review and meta-analysis has been carried out by Martins *et al.* [23], where natural antimicrobial phenolic compounds were

Part of pomegranate used	Form	Test organisms	MICs	Reference
	PomElla® (30% punicalagin)	*Streptococcus mutans,* *Fusobacterium nucleatum,* *Aggregatibacter actinomycetomcomitans,* *Prevotella intermedia*	0.8 μg/mL 0.2 μg/mL 0.2 μg/mL 0.8 μg/mL	[16]
Peel	Acetonic, Methanolic, Ethanolic PPE and Hydro-alcoholic PPE	*Streptococcus mutans,* *Gemella morbillorum,* *Enterococcus faecalis,* *Staphylococcus epidermis,* *Klebsiella oxytoca,* *Enterobacter bugandensis,* *R. dentocariosa,* *Streptococcus mutans*	0.0125–0.025 mg/mL 12.5–25 mg/mL 0.0125–0.025 mg/mL 0.05–0.4 mg/mL 12.5–25 mg/mL 3.15–100 mg/mL 10 mg/mL 10 mg/mL	[17, 18]
Peel, flower, leaf, stem	Aqueous and methanolic	*S. mutans, S. mitis,* *L. acidhopillus*		[19]
Peel	Basic gel formulation including 540 mg pomegranate peel powder	*S. mutans,* *S. mitis,* *S. sanguis,* *C. albicans*	1:16 1:128 1:16 1:64	[20]
	Pomegranate mouthwash (Pomegranate extract were obtained from Verdure science, 30% punicalagin)	*Aggregatibacter actinomycetemcomitans,* *Porphyromonas gingivalis,* *Prevotella intermedia*	62.5 mg/mL >31.25 mg/mL 16.125 mg/mL	[21]
Peel	Methanolic PPE	*S. aureus,* *MRSA,* *E. coli,* *C. albicans*	250 μg/mL 250 μg/mL 250 μg/mL 1000 μg/mL	[15]
Fruit pericarp	Methanolic	*Staphylococcus aureus,* *Streptococcus pyogenes,* *Escherichia coli,* *Klebsiella pneumoniae,* *Proteus vulgaris,* *Pseudomonas aeruginosa*	640–2560 μg/mL 1280–2560 μg/mL 640–2560 μg/mL	[9]
Peel	Standardized extract (13% ellagic acid)	*Propionibacterium acnes,* *Shigella sonnei,* *S. aureus,* *Staphylococcus epidermidis*	15.6 μg/mL 7.81 μg/mL 7.81–15.6 μg/mL 7.81 μg/mL	[14]
Peel	Water	*B. cereus, S. aureus,* *P. fluorescens, S. tyriphium,* *E. coli*	200–450 ppm	[24]
Peel	Acetonic, methanolic, water	*Bacillus cereus,* *Bacillus coagulens,* *B. subtilis,* *Staphylococcus aureus,* *E. coli,* *Pseudomonas aeruginosa*	200–400 ppm 150–500 ppm 200–450 ppm 200–700 ppm 200–400 ppm	[25]
Fruit	Water	*Bacillus cereus,* *E. coli,* *S. aureus*	100 mg/mL 100 mg/mL 100 mg/mL	[27]

Part of pomegranate used	Form	Test organisms	MICs	Reference
Peel	Ethanolic, methanolic	*L. monocytogenes*		[28]

Table 1.
Antibacterial activity of pomegranate extracts against different bacteria.

compared with synthetic antimicrobials by using 16 clinical studies for qualitative analysis, and 12 studies for meta-analysis. For the meta-analysis, six clinical trials were evaluated for the comparison of natural antimicrobial phenolic compounds, including pomegranate extract mouthwash, and synthetic antimicrobials. It was found that natural antimicrobial phenolic compounds are less effective than chlorhexidine for biofilm control, although it showed similar reduction of the oral microbes count which was sub-grouped as total microorganisms, *Streptococcus mutans*, and *Streptococcus spp.* according to type of microorganisms.

Due to its antimicrobial and antioxidant properties, pomegranate extract has been studied for its preservation potential use in the food industry. Kannat *et al.* [24] did a study to evaluate the antimicrobial activity of pomegranate peel against common food spoilers and potential pathogens. It was shown that pomegranate peel extract increased the shelf-life of chicken and meat products and showed antimicrobial activity against to *S. aureus, B. cereus* with a minimum inhibitory concentration at 0.01%. However, it did not show antimicrobial activity for *E. coli* and *S. typhimurium* even at higher concentrations. Other researchers showed that pomegranate extracts were less effective against Gram-negative compared to Gram-positive bacteria, probably due to the differences in cell wall structure [25, 26]. Moreover, pomegranate has been studied in a novel and smart multi-functional hydrogel (MFH) system as a food packaging material since it is easy to monitor of the color change due to changes in conditions such as pH and temperature. The MFH with pomegranate extract showed promising antimicrobial activity on pasteurized milk and cheese over a 7-day period [27]. Pomegranate peel extract was also added in a film formulation to produce a material for food packaging materials with antimicrobial and antioxidant effects. This film formulation was found to restrict the growth of *L. monocytogenes* in pork samples inoculated with this bacterium [28].

The activity of pomegranate extract against bacteria is summarized in **Table 1**.

3. Activity against fungi

Treatment of fungal infections is a big challenge because of the eukaryotic nature of fungal cells that have similarity with host cells. While there are some drugs in the treatment of fungal infections available in the clinic, they are limited and there is a need for new alternatives [29, 30]. There are reports showing antifungal activity of pomegranate extracts, especially against *Candida* species [5, 7, 31], which are part of the normal microbiota of human gastrointestinal, oral, and vaginal mucosae. However, they can cause superficial infections and especially in immunosuppressed patients, they can cause severe infectious problems. In one study, punicalagin showed superior antifungal activity than the conventional fluconazole in an *in vitro* time-kill assay. In addition, punicalagin caused a significant change in *Candida* morphology, and alteration in budding pattern and pseudo hyphae when yeasts were treated with a sub-inhibitory concentration of punicalagin [32]. In another study, pomegranate

extract showed superior inhibitory action against *C. tropicalis* while fluconazole and voriconazole, which are commonly prescribed azoles for fungal infections, have been ineffective against *C. tropicalis* [33].

The potential of pomegranate extract has been studied against fungi in *in vitro* biofilm assays. Microbes in biofilms have substantially different characteristics to those of their free-living planktonic counterparts [34, 35]. In particular, microbes in biofilms are concealed and therefore protected from antifungal agents and plant extracts [36, 37]. Pomegranate extract and its one of the major components ellagic acid were shown to exert a reduction in biofilm formation and eradicated pre-formed biofilm of *C. albicans* in an *in vitro* biofilm study [15]. Spray-dried microparticles containing pomegranate extract showed antifungal activity in *in vitro* assays under both planktonic and biofilm conditions [38]. In addition, the inhibitory effect of pomegranate extract on the growth of *Candida albicans* was demonstrated in an *in vivo* study [31]. In another study, similar effects have been obtained against to *Candida mycoderma* using different parts of pomegranate, fresh fruit and sterile juice [39].

In addition to *Candida* species, pomegranate extracts showed inhibitory activity against dermatophytes, which are fungi which use keratin as a source of nutrition and may cause infection in keratinized tissue parts such as nails, skin and hair follicles. Pomegranate peel extract and punicalagin exerted potent antifungal activity against to *T. rubrum* (125 μg/mL), *T. mentagrophytes* (125 μg/mL), *M. canis* (250 μg/mL) and *M. gypseum* (250 μg/mL). Punicalagin at a concentration of 62.5 μg/mL also inhibited *T. rubrum* spore germination, and it was further found that punicalagin 62.5 μg/mL and nystatin 0.78 μg/mL showed similar inhibition in hyphal growth of *T. rubrum* [40]. Moreover, pomegranate extracts have been researched for use as natural preservatives due to their antifungal (in addition to antibacterial) activities [41]. Pomegranate peel extract showed inhibition activity against to *Aspergillus*

Part of pomegranate used	**Form**	**Test organisms**	**MICs**	**Reference**
Peel	Crude extract, Aqueous fraction, ethyl acetate fraction, butanol fraction, punicalagin	*C. albicans, C. parapsilosis*	3.9–7.8 μg/mL, 1.9–15.6 μg/mL	[32]
Peel	Methanolic	*C. albicans*	> 1000 μg/mL	[15]
Peel	Crude extract, Crude extract in a spray-dried microparticle formulation	*C. albicans, C. parapsilosis, C. tropicalis*	3.9–15.6 μg/mL	[38]
Peel	Hydroalcoholic	*Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum gypseum, Microsporum canis*	125 g/mL, 125 g/mL, 250 g/mL, 250 g/mL	[40]
Peel	Aqueous	*Aspergillus niger, Aspergillus parasiticus*		[42]
Peel	Aqueous	*A. alternata, S. botryosum, Fusarium spp.*		[43]

Table 2.
Antifungal activity of pomegranate extracts against different fungi.

niger and *Aspergillus parasiticus* in a zone of inhibition assays [42]. Pomegranate extract showed inhibitory effects against fungal pathogens which are responsible for fruit and vegetable decay. Punicalagin was proposed as the main compound in the extracts providing the observed antifungal activity and it has been found effective in mycelial growth inhibition against phytopathogenic filamentous fungi such as *Fusarium vertillicoides, Mucor indicus, Penicillium citrinum, Rhizopus oryzae* and *Trichoderma recei* [43]. Also, the growth rate of pathogens presented a negative correlation with total punicalagin content, and it has thus been suggested that pure punicalagin may be used as a control agent in storage disease to prevent the excessive use of synthetic fungicides [44, 45].

The activity of pomegranate extract against fungal microbes is summarized in **Table 2**.

4. Activity against viruses

Pomegranate extracts have been examined as an alternative treatment for viral infections [46–48]. A number of studies have shown that polyphenolic compounds have broad-spectrum antiviral activity, by inhibiting viral DNA and RNA, and directly binding the viral particles. It has also been suggested that polyphenols could provide antiviral activity during intracellular replication [49–52].

Pomegranate peel extract showed antiviral activity against the influenza virus. In a study by Sundararajan *et al.* [53], complete inactivation of influenza virus was observed with 1600 μg/mL pomegranate polyphenols, and 400 μg/mL of same extract showed 99% or more titer reduction in only 5 minutes treatment. This result was similar to another study which showed complete inactivation of H3N2 influenza virus within 30 minutes of treatment and a significant viral reduction with approximately 1 μg/mL pomegranate polyphenols. An *in vitro* study, showed that pomegranate polyphenol extract inhibited viral replication in addition to its virucidal effect – they also obtained same activity for punicalagin and suggested punicalagin is the main compound in pomegranate extract for antiviral activity [54]. In an *in vivo* mouse model study, pomegranate polyphenols applied to the lung were found to reduce influenza infection, without toxic effect to the host [55, 56].

Hepatitis C virus (HCV) is the main factor in end-stage liver disease and approximately 170 million people are chronically infected with HCV. Pomegranate ellagitannins, punicalagin, punicalin and ellagic acid, blocked and inhibited the NS3/4A protease which is a viral polyprotein responsible for processing and replication in HCV. Moreover, punicalagin and punicalin significantly decreased the HCV replication in an *in vitro* cell culture system [57]. The more prevalent adenovirus (ADV) in Hep-2 host cells has also shown susceptibility to pomegranate crude extract, fractions, and main phenolic compounds. It has been found that a n-butanol fraction of pomegranate peel extract and gallic acid showed the highest antiviral activity against ADV. Furthermore, the crude extract, n-butanol fraction and gallic acid inhibited ADV replication in the post-adsorption phase [58].

Herpes simplex virus (HSV) is from the *Herpes* viridae family and infects a high proportion of the populous. HSV-1 is generally responsible for cold sores and encephalitis, whereas HSV-2 is the main causative agent of anogenital infections, which can also infect neonates via the mother [59, 60]. Pomegranate rind extract (PRE) and its major ellagitannin compound, punicalagin, showed virucidal activity against HSV-1. While punicalagin has greater virucidal activity than an equivalent mass of pomegranate rind extract, PRE showed better antiviral activity than punicalagin. Moreover, PRE demonstrated comparable activity to acyclovir against HSV-1 and

Part of pomegranate used	Form	Test organisms	Mechanism of virus target	Reference
Rind	Crude hydraulic extract, Punicalagin, Ellagic acid	Herpes simplex virus	Virucidal activity	[48]
Juice, peel and pomegranate liquid extract		Influenze A viruses, H1N1, H3N2, H5N1 and coronavirus MHV A59	Damage to virion integrity and virucidal activity	[53]
Juice	Pomegranate polyphenol extract, punicalagin, pomegranate liquid extract (from POM Wonderful)	Human influenza A (H3N2)	Inhibition of viral RNA replication	[54]
Peel	Methanolic crude extract, punicalin, punicalagin, ellagic acid	Hepatitis C virus		[57]
Peel	Methanolic crude extract, ellagic acid, punicalagin, gallic acid	Adenovirus	Inhibition of adenovirus replication	[58]

Table 3.
Antiviral activity of pomegranate extracts against different viruses.

HSV-2, in addition to antiviral activity against acyclovir-resistant HSV-1 [48]. PRE is thus a promising new alternative treatment for HSV-1 since currently acyclovir is the gold standard treatment in HSV infections [61].

Studies have suggested that the antiviral activity of pomegranate extract originates from its hydrolysable tannins and polyphenols, especially punicalagin and gallagic acid. However, in one study, four flavonoids, ellagic acid, caffeic acid, luteolin and punicalagin, from pomegranate peel extract were studied against influenza virus and only punicalagin showed an inhibitory effect. The antiviral activity of pomegranate rind extract has been patented in Japan based on pomegranate peel extract ability to prevent the growth and kill viruses on the surfaces [46, 47]. The activity of pomegranate extract against viruses is summarized in **Table 3**.

5. Activity against parasites

Parasitic infections remain a significant global problem, affecting the health of hundreds of millions of people annually, especially in countries with low economic and social conditions. In addition, the increased world-wide resistance to conventional drugs is making most of currently used drugs less effective. As a result of this situation, the development of new drugs from medicinal plants for parasites is as important as for other microbes [62]. Different parts of *Punica granatum* L., root, stem bark, and rind of fruit, have been used commonly as vermifugal and taenicide agents [63]. The antiprotozoal activity of the pomegranate has been determined and in folkloric medicine, it has been used as anthelminthic especially against tapeworms and for diarrhea [64, 65]. A methanolic extract of pomegranate leaves showed nematicide activity and hepatoprotective activity against carbon tetrachloride induced hepatoxicity [66]. Extracts of pomegranate showed anti-schistosomal activity against *Shistosoma mansoni* in both *in vitro* and *in vivo* conditions [67].

Part of pomegranate used	Form	Mechanism of organism target	Organisms	Reference
Seed	Methanolic	Reduction in gastrointestinal motility		[65]
Leave	Methanolic	Larvicidal and ovicidal activity	*M. incognita, R. reniformis, P. penetrans, S. rolfsii*	[66]
Peels, juice and leaves	Methanolic	Reduction in viability of parasite	*Schistosoma mansoni, schistosomules*	[67]
Leaf, stem bark	Ethanolic	worms separation, reduction of motor activity, lethality, and ultrastructural tegumental alterations	*Schistosoma mansoni*	[68]
Peel	Powder form directly given to animal *in in vivo* study	Growth inhibition and death	*Cryptos poridium*	[72]
Juice	Crude extract was applied by patients in a clinical study		*Trichomoniasis vaginalis*	[73]

Table 4.
Antiparasitic activity of pomegranate extracts against different parasites.

In addition, it caused reduction or complete loss of motor activity, lethality and ultra-morphological changes in adult worms [68]. There is thus potential for the treatment of schistosomiasis.

Al-Musayeib *et al.* reported the antiparasitic activity of pomegranate rind extract against *Plasmodium falciparum* [69]. Pomegranate juice was found to exert dose-dependent activity against *Leishmania major* promastigotes and, at >80 μL/mL, gave significantly greater reduction than the positive control, Pentostam. Furthermore, mice that were orally treated with pomegranate juice, showed significantly reduced cutaneous leishmaniasis lesions compared to untreated mice [70]. Calzada *et al*. demonstrated pomegranate antiprotozoal activity against *Entameoba histolytica* and *Giardia lamblia* that cause diarrheic dysentery [71]. Pomegranate peel suspension also affected *C. parvum* in different stages and finally caused parasite death in an *in vivo* murine model; furthermore, pomegranate suspension did not cause any negative change in the mice ileal tissue [72]. In another study, pomegranate extract showed activity against *T. vaginalis*, both *in vitro* and clinically. Patients with *T. vaginalis* infection were treated with pomegranate juice and symptoms were found to have cleared after two months [73]. The activity of pomegranate extract against parasites is summarized in **Table 4**.

6. Potential mechanisms of antimicrobial activity of pomegranate extracts

From the preceding sections it is clear that there is compelling evidence demonstrating the broad-spectrum antimicrobial activity of pomegranate extracts [74–76]. However, the precise mechanism behind this activity is not fully

understood. The mode of antimicrobial action of polyphenols, in general, is also unknown, although some suggested mechanisms include membrane disruption, toxicity against microorganisms, the ability of complex formation with metal ions and enzyme inactivation [77–79]. The antimicrobial activity of pomegranate has been associated with polyphenolic tannins, especially punicalagin and ellagic acid content in the extract [80–82]. However, pomegranate extracts are a complex mixture containing a variety of secondary compounds and interplay between these components may be a factor in antimicrobial activity, with multiple mechanisms operating independently [83].

An antimicrobial mechanism suggested for polyphenolic compounds is based on the precipitation ability of these compounds with bacterial cell membrane proteins which leads to bacterial cell lysis [84]. In addition, polyphenols could inhibit microbial enzymes by reacting with sulfhydryl groups or nonspecific interactions with proteins [85]. In that vein, phenolic compounds can bind the protein sulfhydryl groups and make them unavailable for microbial growth [86]. In addition, it has been reported that polyphenols can damage the microbe respiratory chain by decreasing the oxygen consumption and thus limiting the oxidation of NADH [87].

It has been hypothesized that the antibacterial activity of phenolic acids and flavonoids could cause a decrease in membrane fluidity by giving damage to the bacterial cytoplasmic membrane [88]. Phenolic acids can cause hyper acidification when they interphase with the plasma membrane. This situation would cause an alteration in cell membrane by making it more permeable. This mechanism could explain why phenolic acids show different antimicrobial activity levels against different pathogenic microorganisms [89, 90]. One of the possible mechanisms could be related to hydroxyl groups of polyphenols. The position of OH group in the aromatic ring and the length of saturated side chain could be a cause of antimicrobial activity of polyphenols [91]. Hydroxyl groups can bind to bacteria cell membranes and interfere with processes, such as ion pumping. In addition, OH groups can interact with active site of enzymes and disturb the metabolism of microorganisms [91].

Pomegranate extract exerted an inhibition activity against biofilms, in addition to their planktonic counterparts. Since microbes act differently under biofilm conditions compared to their planktonic form, there are some suggested pathways about polyphenols biofilm eradication and formation inhibition activities, although still unconfirmed. The mechanism behind growth and biofilm inhibition by pomegranate extracts cause protein precipitation and enzyme inactivation [81, 92]. Pomegranate extract could precipitate proteins which play major role in biofilm formation, like adhesins. Moreover, major hydrolysable tannins in pomegranate extract such as ellagic acid can change the surface charge and reduce the cell-substratum interactions and biofilm formation and development on different surfaces [93]. It is well known that tannins have astringency properties, and this feature can play a part in biofilm disruption [94, 95]. Different studies have shown the activity of pomegranate on bacterial attachment and therefore biofilm formation. It has been demonstrated that *Punica granatum* L. showed a specific antimicrobial action on dental plaque, which is a complex biofilm on tooth, by inhibiting adherence mechanism of oral microbes to dental surface via disturbing polyglucan synthesis [17, 96, 97]. Moreover, Vasconcelos *et al*. [98] used *Punica granatum* L. in a gel formulation using increasing and doubled concentrations of the diluted solutions of the gel with ranging concentrations from 1:1 to 1:1024, and similar results obtained. The gel formulation inhibited the adherence of different bacterial strains and a yeast, *C. albicans*, in the oral cavity and affected preformed biofilm.

There are some reports suggesting that the inhibition of quorum sensing (QS) could play role in the biofilm inhibition activity of pomegranate [99, 100]. QS is a communication system between bacteria in a biofilm, and provides a network

involving nutrients, defense against other microorganisms, virulence and biofilm formation. More importantly, QS helps microbes to escape from host immune response [101, 102]. Therefore, inhibition of QS is quite important in order to overcome microbial infectious diseases and resistant pathogenic microbes. For the evaluation of QS inhibitors, *Chromobacterium violaceum* has been used as a biosensor since it produces violacein, purple pigment color, in response to QS regulation [103]. Pomegranate inhibited the QS of two bacterial strains which are *Chromobacterium violaceum* (by affecting purple pigment production) and *P. aeruginosa* (by decreasing bacterial swarming motility) [104, 105]. In another study, different compounds from herbs, fruits and plant extracts have been studied for their QS activity, with resveratrol and pomegranate extract demonstrating the highest inhibition activities. The QS activity of pomegranate has been associated with ellagic acid content of pomegranate extract (85% punicalagin, 7% free ellagic acid) since ellagic acid showed 86% inhibition at a low concentration of 4 μg/mL. However, the anti-QS activity of punicalagin is also believed important in pomegranate extracts [106]. Tannin-rich fraction of pomegranate rind extract showed inhibition of biofilm formation and motility of *E. coli* and repressed the expressions of curli genes (*csgB* and *csgD*) and various motility genes (*fimA, fimH, flhD, motB, qseB*, and *qseC*) [107]. Similarly, punicalagin significantly decreased the expression of QS-related genes (*sdiA* and *srgE*) of Salmonella [108].

The chemical structure of tannins has importance in bacterial growth inhibition. For example, hydrolysable tannins were found to give lower minimum inhibitory concentration than condensed tannins [109]. The degree of galloylation has an effect on antibacterial activity since a higher degree of galloylation have more protein binding capacity and higher affinity for iron. However, the antibacterial activity is not only correlated to galloyl groups and galloylation, also it is correlated to configuration of the digalloyl or trigalloyl groups that attached to glucose core [110–112]. In addition, free galloyl groups have a major role in antimicrobial activity of ellagitannins which are abundant secondary compounds in pomegranate extracts [12, 113]. Punicalagin showed the broad-spectrum antimicrobial activity and it has a gallagyl moiety [114]. However another ellgitannin, granatin A, which does not have free galloyl groups, did not show antibacterial activity [115]. In a study done by Reddy *et al.*, ellagic acid, gallagic acid, punicalin and punicalagin were purified and tested for their antiplasmodial and antimicrobial activities. Gallagic acid and punicalagin showed the strongest effects on the growth

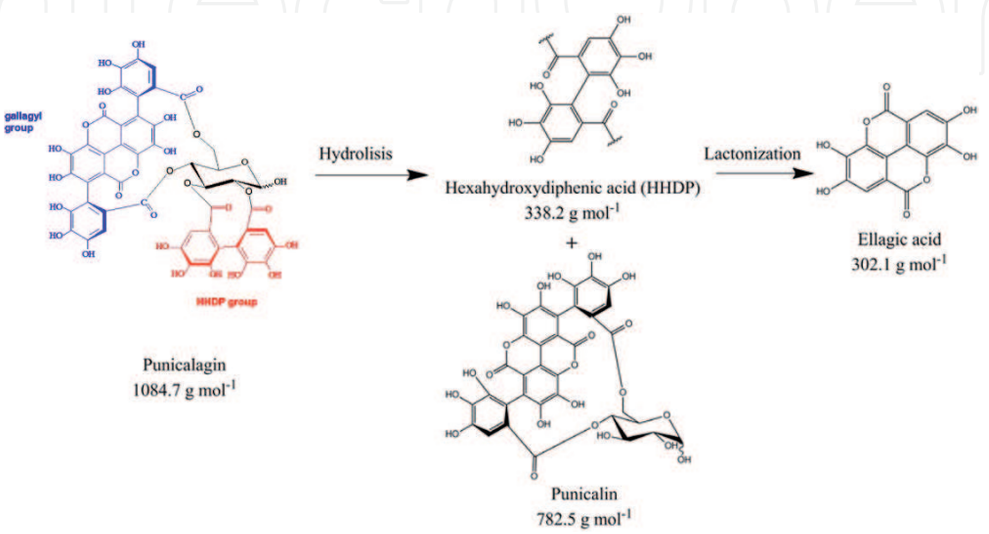

Figure 1.
Reduction of punicalagin, punicalin and HHDP to ellagic acid, adopted from Seeram et al. *[3, 12].*

of bacteria and fungi and it has been suggested that the ellagic acid moiety is not important compared to the gallagyl and hexahydroxydiphenol (HHDP) moieties for the inhibition of microbes [116]. The degradation of punicalagin to ellagic acid, via punicalin and hexahydroxydiphenic acid is shown in **Figure 1**.

The antimicrobial activity of plants has been studied extensively and many active secondary compounds have been identified. However, it should not be ignored that plant extracts with antimicrobial activities contain potentially many secondary compounds. Therefore, it is not easy to attribute the biological activity of plant extracts to only a single compound or a group of secondary compounds. There is a high possibility that plant extracts show antimicrobial activity due to synergistic effect of different compounds [117].

7. Enhanced antimicrobial activity of pomegranate extracts with metal ions

There are many reports showing the antimicrobial activity of heavy metals such as iron, copper, silver, manganese and zinc, while many bacteria have mechanism for the detoxification of heavy metals [118, 119]. However, although metal ions have a strong antimicrobial effect, they can also be cytotoxic to human cells, therefore, the use of these metals may have limitations in healthcare [120, 121].

Stewart *et al*. [122] investigated the potentiated antimicrobial activity of pomegranate rind extract (PRE) in combination with metal ions. In their study, the aim was to exert short term exposure of pomegranate rind extract and ferrous sulfate combination on bacteriophage levels for 3 minutes. This combination showed highly significant synergistic activity and reduced the bacteriophage levels in a short-term exposure. This short screening time was necessary for this experiment due to low stability of pomegranate rind extract/ferrous salt solution which, via a Fenton reaction caused Fe^{2+} to oxidize to Fe^{3+} with concomitant solution blackening. To overcome this instability problem, potentiated/synergised antimicrobial activity of pomegranate rind extract has since been examined using alternative metal ions [48, 123, 124].

McCarell *et al.* [123] investigated the antimicrobial activity of PRE with 4.6 mM $FeSO_4$, $CuSO_4$, $MnSO_4$, ZnO and also studied antimicrobial activity of PRE/metal salt combinations plus vitamin C which was added as a stabilizer. They observed significant synergistic antibacterial activity against *E. coli, Ps. Aeruginosa, S. aureus* and *P. mirabilis* when they combined PRE with Cu (II) ions. Moreover, with the addition of vitamin C as antioxidant, the antimicrobial activity increased significantly for PRE/Fe (II) and PRE/Cu (II) combinations against *S. aureus*. In another study, researchers used the vanillin complexes with different metal ions using the agar diffusion method and it was found that the vanillin and metal salts showed an enhanced activity against *S. aureus, E. coli, K. pneumanie, P. aeruginosa and C. albicans*. The results from both studies indicated that the addition of metal ions, especially copper salts, can significantly enhance antibacterial activity of a natural product [123, 125].

Significantly enhanced virucidal activity of PRE was later observed against HSV-1, HSV-2 and acyclovir-resistant HSV-1 by Houston *et al.* [48] in combination with different Zn (II) ion salts, including zinc sulphate, zinc citrate, zinc stearate and zinc gluconate, with a maximum of 6 log reduction observed. Unlike PRE and Fe^{2+}, this activity was not time-limited, and was not associated with blackening. Importantly, this activity was also retained when applied to epithelial surfaces prone to *Herpes* infection, including buccal and vaginal mucosae [126], indicating potential treatment for cold sores and anogenital *Herpes*.

The mechanism for the synergistic antimicrobial activity of pomegranate extract in combination with metal ions is not clear, although there are some putative suggested mechanisms for this enhanced antimicrobial activity. For instance, it has been suggested that pomegranate tannins can form a 'complex' with metallic ions and this complex could show enhanced toxicity to microbes [127]. Furthermore, PRE could show enhanced activity due to redox cycling of the associated metal ion which increases local levels of reactive oxygen species (ROS). For example some antibiotics e.g. bleomycin showed enhanced ROS production via the ability to bind to ferrous ions which resulted in enhanced toxicity against microbes [128].

The enhancement of antimicrobial activity of pomegranate rind extract with metal ions is important in terms of improved efficacy against antibiotic resistant pathogens, since this enhancement could reduce resistance of microbes by inhibiting their microbial adaptability features [8, 32].

8. Conclusions

The pomegranate has a long history of use as a folklore medicine for its ability to address microbial infections. Published research, as outlined in this chapter, clearly supports this and has shown that pomegranate extracts possess an unusual and potent broad-spectrum of activities against bacteria, fungi, viruses and parasites.

There is some variation in the literature in terms of the levels of antimicrobial activity of pomegranate extracts, which could be attributed to different harvesting time and type of pomegranate cultivars, and use of varying microbial strains. However, it is also apparent that different workers have used a range of approaches to obtain 'pomegranate extract', with extraction methods sometimes being poorly described. As such, a lack of standardized test extracts presents a challenge in attempting to make quantitative comparisons. As a complex mixture, pomegranates extracts have the innate ability to inhibit resistance, even more so when used alongside a synergizing metal ion.

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Conflict of interest

The authors declare no conflict of interest.

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Author details

Vildan Celiksoy and Charles M. Heard*
School of Pharmacy and Pharmaceutical Sciences, Cardiff University,
United Kingdom

*Address all correspondence to: heard@cardiff.ac.uk

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